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THE NATURAL HISTORY OF THE NINE- BANDED ARMADILLO OF TEXAS

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For some years past the writer has been engaged in a study of various phases of the biology of the Texas armadillo and has published a number of papers, some of them in collaboration with J. T. Patterson and some of them alone, dealing with matters of development, cytology, sex and heredity. There now appears to be a demand for a brief, non-technical summary, giving the gist of the findings discussed in detail in these papers. The present account will furnish such a summary and will in addition deal with certain matters not yet published.

NOMENCLATURE AND AFFINITIES

In the publications thus far issued the armadillo of Texas has been referred to under various generic titles (*Dasypus*, *Tatusia* and *Tatu*) and it would be well to come to a final decision as to nomenclature. The systematists seem to have finally settled upon the name *Dasypus novemcinctus texanus*. They recognize two other subspecies of this form in North America, viz., *D. novemcinctus fenestratus*, the common Mexican armadillo, and *D. novemcinctus hoplites*, a type described by Allen from the hills of Grenada. These three subspecies are probably no more than local varieties of which many others could no doubt be discovered were one inclined to make

a careful survey of the range of the species. There is scarcely a doubt that the North American armadillos are all derivatives of the Pebas armadillo (*Dasypus novemcinctus*) of South America, a species of wide range, occurring from Panama to Paraguay. The mulita of the Argentine and Kappler's armadillo of Surinam were formerly classified as species of *Dasypus*, but the former is now *Cryptophractus hybridus* and the latter *Tatusia kappleri*. Nothing is known about the development of the latter, but the preliminary paper of Fernandez shows that the mulita is strikingly like our species in the details of polyembryonic development. Such a fundamental resemblance would seem to indicate that the two species are very closely related and should be classed in the same genus. About a dozen other species of armadillo, assigned to several other genera, are native to South America. About their natural history little is known.

RANGE, DISTRIBUTION AND FUTURE OF THE ARMADILLO IN TEXAS

In his "Biological Survey of Texas" Bailey (1905) states that

The armadillos are strictly Lower Sonoran, but in the rough country between Rock Springs and Kerrville they range fairly into the edge of the Upper Sonoran Zone. As a rule they do not extend east of the semiarid or mesquite region, nor to any extent into the extremely arid region west of the Pecos, but occupy approximately the semiarid Lower Sonoran region of Texas north to near latitude 33°.

Bailey lists many localities from which armadillos have been taken or authentically reported. To this list I should like to add the following localities, which I have visited and from which I have obtained considerable numbers of specimens: Boerne (over 100), Comfort (nearly 200), Fredericksberg (about 40), Kerrville (about 25), Ingram (90), Helotes (3). Many of those reported from Boerne, Comfort and Ingram were brought from distances of twenty miles or more. At the towns of Boerne and Comfort we find a flourishing

industry in which the armadillo furnishes the raw material. Many thousands of the adult animals are slaughtered annually for their armor, which is shaped into baskets and sold all over the world as curios. Armadillo hurting has come to be a recreation and a source of additional income for large numbers of young American and Mexican farmers. When they come to town to sell produce and purchase supplies they bring also many armadillo baskets which they have learned to make in an expert fashion and for which there is a ready market. One dealer with whom I am well acquainted claims to have shipped no less than 40,000 baskets during the last six years. At least two other firms have been almost equally active. In spite of this extensive slaughter the animals seem to be increasing in numbers, for I had no difficulty in obtaining in about two weeks nearly two hundred pregnant females. Those used in my work would have been slaughtered for their armor alone, so I felt no compunction about destroying so many unborn young. Hunters and dealers generally have the idea that the range of the armadillo is extending rapidly

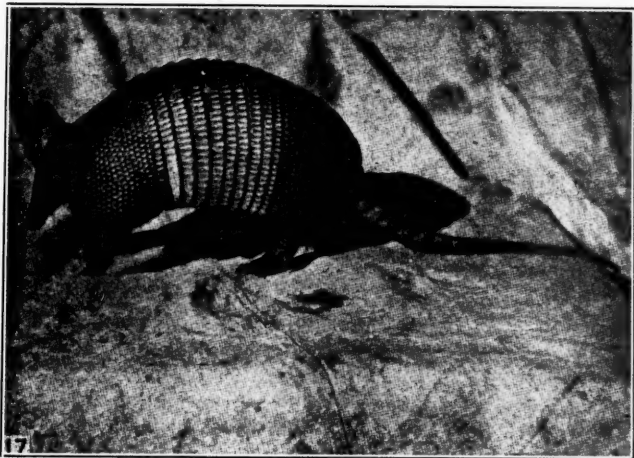


FIG. 1. Photograph of a living armadillo showing the complete armor and the usual resting attitude. The head is usually withdrawn between the two flaps of the shoulder shields when the animal is feeding in the thickets.

northward and eastward. There seems to be no reason to doubt that the species is multiplying and spreading, for I have it on good authority that in the regions where it is now most plentiful it was almost unknown 20 years ago. Its range is, however, strictly circumscribed by definite ecological conditions as I shall proceed to show.

ECOLOGY AND HABITS

The armadillo spends its life on the defensive and its defensive equipment consists of structural and functional adjustments to a very special environment. Of the structural adaptations the armor (Fig. 1) is the most obvious, but its use is not what it is commonly supposed to be. While the carapace doubtless serves partially to protect the animal when it is attacked by large carnivores, the fact that dogs often bite through the bony plates and seriously damage the shell shows that for this type of enemy the protection is very inadequate. In fact it is the experience of hunters that, when closely pressed by dogs, the harassed animal turns on the back and strikes most effectively with the powerful claws. The armor has a much more important significance in that it enables the animal to invade the dense, thorny thickets of cactus and chapparral, etc., that characterize its normal habitat. When pursued it is possible for the armadillo to plunge headlong into a mass of thorny vegetation that would be totally inaccessible for an unarmed enemy. Then too they can penetrate all sorts of underbrush in search of insect food without danger from thorns or spines. In some regions of the country the animals take advantage of the rough and rocky character of the hillsides and river banks, seeking shelter from enemies by retreating into holes and crevices among the rocks that are just large enough to admit them but too small for their enemies. Armadillos living in these regions have the armor much worn from rubbing and scraping against the angles and sharp edges encountered in the rocky passages of their retreats. If one is able to

reach the tail of an armadillo concealed in a rock pile the animal braces the armored back against the roof or sides of the hole and holds so hard that the tail will come off before the body can be moved. Thus in divers ways the armor serves a protective function other than the primary one connoted by the name. Still further, there can be no doubt but that the carapace serves as a reducer of surface evaporation, an important factor in making life possible in the semiarid regions, for there are many periods of extreme drought during which it must be of vital importance to conserve moisture. It is possible, indeed probable, that the armor is phylogenetically older than the particular conditions comprising the present environment of the armadillo, hence we can scarcely claim that the armor is in any strict sense an adaptation. It seems far more likely that in the exercise of its prerogative of choice of habitat the species has selected an environment affording an unpreempted food area and an adequate shelter from enemies.

The armadillo is preeminently insectivorous, although in captivity it appears practically omnivorous. Stomach examinations of freshly caught wild animals show the remains of insects, chiefly ants, together with much earth and more or less vegetation. In captivity they eat meat of all kinds, even exhibiting cannibalistic propensities under certain conditions, for when shipped in crates or boxes the stronger ones kill and disembowel the weaker, and mothers devour their own new-born offspring. Hunters and basket dealers justify the extensive slaughter of the armadillo by giving to the animal a bad name. It is said, on how good authority I am unable to state, that the "'dillo" is a robber of newly made graves and a destroyer of vast numbers of the eggs of such ground birds as wild turkeys and quails. They are also said seriously to damage the grazing value of certain territories by rooting up quantities of grass. I am of the opinion that much of the destruction of bird eggs and of grass might more justly be blamed upon the Texas

peccary, which has a range quite similar to that of *Dasypus*.

Armadillos are essentially nocturnal in habit, although one may encounter them at dusk. On warm nights they spend their time rooting about in the dry leaves and ground vegetation after the manner of hogs. Their grunting, snuffing noises are heard at some distances on quiet evenings. The strong burrowing claws are used to a considerable extent in digging for food, but their primary function is that of burrowing. Burrows may be for temporary or permanent shelter. A permanent burrow may be dug six or seven feet deep with a chamber at the bottom about two feet in diameter, which is filled loosely with dried leaves and grass. This is the winter retreat of the armadillo, where he undergoes partial hibernation during the periodic cold spells. Buried in the grass and leaves, the animal defies its worst enemy, cold. In this connection it may be said that there is probably no mammal so sensitive to cold as the armadillo. In captivity they shiver at temperatures when other mammals are warm, and often die during the night if insufficiently bedded down with straw. Their further spread northward will no doubt be blocked by temperature barriers. Temporary burrows are made as a retreat from enemies when other shelter is unavailable. Hunters claim that an armadillo will dig a hole in ordinary soil in a minute or two, disappearing even after having been sighted.

They seem to have their regular haunts and do not ordinarily go far from their burrows or caves. From the smoothly worn mouths of these retreats beaten paths lead to thickets, pools and streams. Bailey has seen evidences that they, after the manner of pigs, enjoy a mud bath. The trail of the tail along the paths is a ready means of distinguishing the haunts of the "'dillo," for it leaves a mark like that of a dragging rope.

In captivity the animals display the utmost gentleness and tractability so long as one does not attempt to

lay hands upon them. If one attempts to hold one of them by the shell he will realize how strong and active is its resistance, for it bucks vigorously like a broncho and throws off all holds. The tail is the weak point in its defence, as it offers a perfect handhold, but, even when grasped by the tail, it furnishes an interesting struggle by violently rotating the body and often succeeds in twisting free from the enemy's grasp. Then one is surprised at the speed of which the animal is capable, its galloping gait being apparently unhindered by its armored cuirass.

Of the senses, that of smell is the only one upon which the animals seem to rely. When feeding they frequently raise the snout on high and sniff the air in all directions. The eyes are rudimentary and practically useless. If disturbed an armadillo will charge off in a straight line and is as apt to run into a tree trunk as to avoid it. That the hearing is not at all keen is evidenced by the fact that one may approach them on the leeward side even if the approach is somewhat noisy.

BREEDING HABITS

Information as to mating and care of young has come indirectly through hunters, among whom there is a considerable degree of consensus of statement. It is claimed that armadillos pair for life or at least for the season. It is very common to capture a male and a female together or to dig a pair out of a burrow. The period of œstus comes early in the autumn, extending over a variable period of time. A large proportion of the females taken in October show the early stages of pregnancy, but early stages have been found as late as December. It seems probable that the young "does" of the previous season's crop reach maturity late in the autumn, for the largest females are almost invariably pregnant in October while many of the smaller females are non-pregnant at that time. The young are for the most part born in March, although births during April are not rare.

From these observations it may be estimated that the period of gestation averages from the middle of October to the middle of March, a period of five months or 150 days, an extended gestation period for so small a mammal. The young are fully formed at birth, with eyes open and with a complete though not very hard armor. They are able to walk in a more or less uncertain fashion within a few hours after birth.

Copulation occurs with the female turned on the back, this position being necessary on account of the armor and the ventral location of the genitalia.

POLYEMBRYONIC DEVELOPMENT

Our earliest observations dealing with the development of the Texas armadillo revealed the facts that the four embryos are enclosed in a common chorion and that these monochorial quadruplets are always unisexual. These early observations stimulated an investigation of the embryological and cytological conditions that underlie polyembryony and sex-determination. The published accounts carry the history of development through the period of ovogenesis up to the time of fertilization and from the primitive streak stage to birth. The hiatus between fertilization and the formation of the primitive streak is almost completely filled by two sets of observations, one by Patterson, who has secured late cleavage stages and all of the history up to the primitive streak, and the other by the writer, who has described the early cleavage of parthenogenetically developing ova. The observations of Patterson were reported at a meeting of the central branch of the American Society of Zoologists at Urbana in 1912; the paper on parthenogenetic cleavage is now in press and will no doubt appear before the present contribution. By piecing together the subject matter of these separate investigations the writer is able to offer the following account of the development.

OVOGENESIS AND FERTILIZATION

The early phases of ovogenesis are in no way peculiar and in themselves offer no clue as to the physiology of polyembryonic development. A detailed study of the growth period of the ovocytes and of folliculogenesis shows that in normal ovaries there is only one ovocyte to the follicle and that in ovulation only one egg is given off at a time. The details of maturation are like those of other mammals, especially like those of the marsupial *Dasyurus* as presented by Hill ('10). The growth period involves an accumulation of deutoplasmic material, which in the full-grown ovocyte lies in the form of a coarsely vacuolated central sphere containing deeply staining granules. Surrounding the deutoplasmic sphere is a fairly thick peripheral zone of homogeneous protoplasm, called the formative zone (Fig. 2), which is somewhat thicker at the animal pole where the germinal vesicle is flattened against the zona pellucida. During the maturation process a remarkable reorganization of the cytoplasmic regions of the ovocyte occurs. The fluid deutoplasmic sphere forces its way to the surface and comes to lie in contact with the periphery of nearly the whole animal hemisphere of the cell. This forces the formative protoplasm to the vegetative pole where it assumes the form of a cap thick at the pole and thin at the equator, having a crescentic outline in meridional section (Fig. 3). The maturation spindle, forced from its normal position at the animal pole, lies as near the latter as possible without leaving the formative protoplasm, and assumes a position tangential to the nearest periphery of the cell, but nearly parallel to the primary axis of the latter.

The two maturation divisions occur without disturbing this new arrangement and no other radical change seems to take place until after fertilization, at least so one must conclude from the fact that a tube egg in a late phase of fertilization still shows the formative and deutoplasmic zones arranged as in Fig. 2. This one fertiliza-

tion stage (Fig. 4) shows two polar bodies and the male and female pronuclei lying close together in the thickest part of the formative zone. There is nothing in maturation nor in fertilization to suggest or account for polyembryony. Their chief evidential value lies in the fact that they demonstrate the fact of polyembryony and show that the latter is not due to any

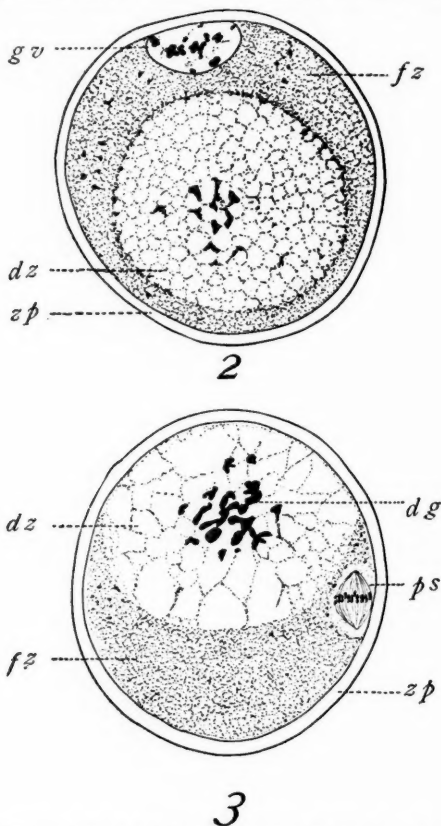


FIG. 2. A section through a full-grown ovocyte before the changes incident to maturation have taken place. Note the peripheral formative zone (*fz*), in which lies the flattened germinal vesicle (*gv*). The deutoplasmic zone or sphere (*dz*) lies in the center. The zona pelucida (*zp*) is a dense shell-like membrane.

FIG. 3. An ovocyte during the first maturation division, showing the reorganized cytoplasmic zones. The polar spindle (*ps*) is situated far from the animal pole. The deutoplasmic granules (*dg*) are conspicuous at this period. Other lettering as in Fig. 2.

morphological peculiarity of the germ cells. In brief the egg is a simple egg with one nucleus and is fertilized by a single spermatozoon. Hence the embryo starts out as a single and not as a multiple individual.

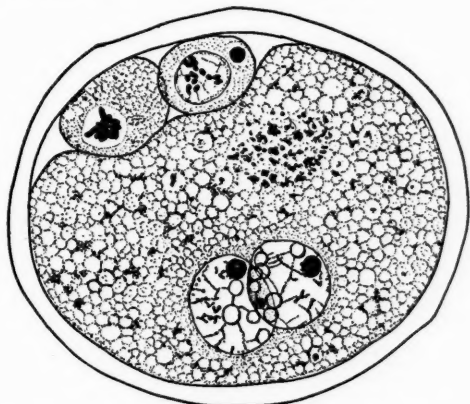


FIG. 4. A fertilized egg found in the fallopian tube, showing the male and female pronuclei in contact and occupying the thickest part of the formative protoplasm. There are two polar bodies. The deutoplasmic zone does not appear in this section.

The claim of Rosner ('01), based on an examination of one pair of ovaries inadequately preserved, that the four embryos are the result of the fusion of several follicles and the subsequent fusion of the several eggs or vesicles given off by the rupture of a compound follicle, is completely refuted by the present studies. It may be of interest to show how Rosner came to fall into so serious an error. The writer after the examination of a large number of normal ovaries chanced upon one pair showing substantially the conditions described by Rosner. These ovaries were from a very large, old female and when examined cytologically showed many multiple follicles, containing from two to eight or more oocytes in various stages of development. Everything about these ovaries, however, is atypical and there can be no doubt as to their pathological character. That Rosner should by chance have stumbled upon such an ovary and that he drew a general conclusion as to the normal con-

dition from so slender an evidential basis constitutes a biological comedy of errors scarcely equaled in our literature.

CLEAVAGE

Nothing is at present known of the early cleavage stages of the fertilized egg and I shall offer here as a tentative substitute facts dealing with the parthenogenetic cleavage of eggs in atretic follicles. The first step in the development of such eggs is the elimination of the deutoplasmic material, which probably is thrown out of the protoplasm by a rupture of the plasma membrane of the egg. The formative protoplasm of the egg in this way unburdens itself of a considerable volume of inert and probably deleterious material, which, although outside of the egg-cell proper, remains within the zona pellucida and more or less completely surrounds the egg in the form of pseudo-epithelium of cell-like masses, which I have called cytoids. The egg now consists of a homogeneous, clarified protoplasm and there is every reason to suppose that the elimination of byproducts of metabolism has served to rejuvenate the cell so that its normal processes of growth and reproduction may be resumed. The nucleus, which, previous to and during maturation, had ceased to carry on metabolic exchanges with the cytoplasm, now evinces renewed activity in that astral rays, entirely absent during maturation divisions, now penetrate the entire cytoplasm and a typical cleavage spindle appears. Two-, four- and eight-cell stages occur in fairly regular fashion, but even at the eight-cell stage unmistakable signs of degenerative changes manifest themselves, which bring about a rapid dissolution of embryonic integrity and inhibit further progressive changes. There is no evidence in this material that parthenogenetic development proceeds to the formation of teratoma or tissue formation; in fact, the total lack of cleavage stages later than about the eight-cell stage argues strongly against the possibility of the development of any such structures. This study serves two pur-

poses, that of affording a critical demonstration of parthenogenetic development of mammalian ova, and that of furnishing a clue as to what we may expect to find when we come to know the facts about the early cleavage of normally developing eggs. In the latter connection it is of interest to note that in *Dasyurus*, whose developmental peculiarities up to the time of cleavage parallel those of the armadillo, there is, as a preliminary to cleavage, an elimination of the deutoplasmic material almost precisely like that shown in our parthenogenetic material. This fact lends support to the conjecture that, in essential features, parthenogenetic cleavage parallels that of normal development and may be used as a substitute for the latter, at least up to the eight-cell stage.

For the sake of rendering the present account as nearly complete as possible I shall make a statement regarding the late cleavage and early embryology, based partly on Patterson's observations. The earliest stage shown by the latter at the Urbana meeting was an inner-cell-mass stage, like that of any ordinary mammal. Such a vesicle becomes attached by its animal pole to the very apex of the fundus of the uterus, where it lies in a position predetermined for it at a point where two grooves in the uterine mucosa cross each other, the one running laterally between the openings of the fallopian tubes and the other at right angles from mid-dorsal to mid-ventral aspects of the uterus. This position at the crossing of these grooves enables the investigator to locate with certainty even the excessively minute earliest stages of the developing vesicle. As it expands the vesicle becomes depressed in the groove and elongates laterally into an ovoid form with the long axis running from the right to the left sides of the uterus. As soon as it gains attachment to the uterine mucosa the vesicle undergoes germ-layer inversion like that seen in the rodents, the result being that two secondary vesicles are produced, an inner complete ectodermal vesicle and an outer endodermic vesicle, incomplete at the area of attachment where the primitive

placenta or Träger arises. Up to this time there is no sign of polyembryony. The first step in the direction of a division of the single embryonic vesicle into four embryonic rudiments is seen in connection with mesoderm formation. The mesoderm arises at two points, to wit the extreme right and left sides of the laterally elongated vesicle, and soon assumes the form of two hollow pouches that subsequently expand and fuse together in the median lines into a common extraembryonic body cavity. This mode of origin of the mesoderm shows that the embryo is no longer developing as a unit, but that there has arisen a bilateral duality of function, due probably to the partial physiological isolation of the right and left sides of the mesoderm. The possible cause of this isolation will be discussed presently. The first recognizable rudiments of the embryos appear as two blunt processes or

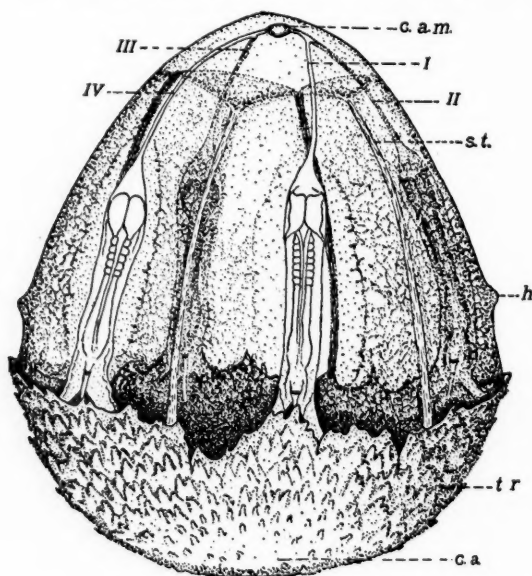


FIG. 5. An early embryonic vesicle showing four embryos enclosed within a common chorionic membrane. The small common amnion (*c. am.*) still retains its connection with the separate amnia of the embryos by means of the slender amniotic connecting canals (I-IV). The four embryos are attached to a common discoid primitive placenta, the Träger (*tr*), by belly-stalk bands.

thickenings of the ectodermal vesicle. These two primary embryonic buds arise in connection with the dual centers of origin of the mesoderm, each appearing directly beneath a primary mesodermal pouch. These two primary buds elongate and soon divide at the tip into paired outgrowths, which constitute the primordia of the two pairs of embryos. The embryos develop on the inside of the inner vesicle and are consequently in a common ectoderm-lined, fluid-filled cavity, which is a sort of common amnion. Subsequently the separate embryos sink into the floor of the common amnion and retain their connection with the latter only by slender amniotic connecting canals, which gradually shrivel up and disappear. An early somite stage with the common amnion and the connecting canals still intact is shown in Fig. 5, which also illustrates the attachment of the four *fœtuses* to the *Träger* by means of the allantois and the belly-stalk bands which constitute the primitive umbilicus. The saucer-shaped *Träger* or primitive placenta develops from the part of the trophoblast which originally formed the point of attachment for the vesicle. This area has not been invaded by the entodermal vesicle, but is reinforced directly by mesoderm, which invades the maternal mucosa and produces primitive villi, that are at first in the form of blunt ridges, but later take on the form of flat scales (see Fig. 5), and subsequently assume the typical arborescent form of definitive placental villi. The subsequent development of the embryos is of little interest except to the specialist and need not be referred to here. The history of the placenta, however, is of unusual interest in that it illustrates the futility of attempting to use the special types of placentation as criteria of animal affinities. The early placenta as shown in Fig. 5 is a single discoid structure. Subsequently the points of attachment of the four umbilical cords become areas of rapid placental development and the parts of the *Träger* in between them almost lose their villi. At this stage the placenta consists of a set of four separate discs. As

these villous regions expand they come into contact at their margins and apparently fuse into a lobate zone, which had been called a compound zonary placenta. Finally the zone separates along the dorsal and ventral lines to form two lateral notched discoid placenta, to which we need scarcely apply a name. It is obvious that there is nothing to be gained by attempting to classify such a placental complex or by comparing it with those of other groups of mammals, for the peculiar conditions seen here are obviously merely very special adjustment to the peculiar conditions arising from polyembryonic development within a single chorion. The *fœtuses* after they have once been separately outlined are distinct, complete units and are associated scarcely more closely than are the embryos of other forms of mammals where several individuals develop simultaneously in a single uterus, for they have their own separate amnia and separate placentation, and there is absolutely no admixture of fetal blood.

Without further burdening the reader with an elaboration of embryonic details and relations we may briefly summarize the situation in-so-far as the question of specific polyembryony is involved. The ovogenesis is normal; a single egg is fertilized by a single spermatozoon; the cleavage is apparently normal and gives rise to a blastodermic vesicle similar to that of other mammals, especially the rodents; germ-layer-inversion affords an easy mechanism for producing several embryos in a single chorion, for the quadruplets arise by means of dichotomous budding of the inner ectodermic vesicle without affecting the enveloping membranes of the vesicle, which form the common chorion; the subsequent embryonic development of the several embryos is as independent as it can be under monochorial conditions, since each individual has its own separate amnion, allantois, umbilicus and placenta. This in brief is the polyembryonic situation, a consideration of which offers for solution several problems peculiar to the material. What are the physio-

logical causes of polyembryony? What factors determine the definite bilateral orientation of the embryos in the vesicle, or what factors are responsible for pairing of embryos? What light does the situation throw on the problem of sex determination? Does the condition give us any fulcrum on the problem of predetermination and epigenesis? What are the modes of inheritance peculiar to polyembryony? Does the polyembryonic situation offer any new facts bearing on the general problems of genetics? These problems will be discussed in the order given.

THE CAUSES OF POLYEMBRYONY

In a previous paper (Newman, '12) were listed a series of seven possible explanations of polyembryony, nearly all of which assumed some abnormality in ovogenesis, maturation or fertilization. The discovery that all of these processes are normal in the armadillo served to eliminate all but the last suggestion, which was to the effect "that the cause of specific polyembryony may lie in factors strictly external to the ovum, among which one of the most probable is in some way associated with the bilaterality of the uterus." At that time no discussion of that possibility was attempted. The discovery of a specific parasite within the armadillo egg, together with a consideration of certain unpublished data presented orally by Patterson, leads me to hazard the following hypothesis.

A careful examination of many ovaries and many thousands of oocytes has revealed the universal presence of what I consider to be a protozoan parasite in the egg cytoplasm. This parasite is a large body as compared with the size of the host cell and must have a deleterious effect on the egg, probably weakening it or lowering its vitality. Such a depressed egg, in which the parasite has grown and multiplied, develops into a vesicle of some size before the effects of a lowered vitality become apparent. When, however, under the pressure exercised by the transverse groove in the uterine mucosa,

the vesicle becomes elongated lateraliy so that its right and left sides come to be separated a maximum distance from each other. In such a depressed and weakened vesicle unity of functioning ceases to exist and two new centers of growth arise at points where the pressure is less severe, viz., the opposite ends of the elongated vesicle. We have seen that mesoderm forms at two lateral points and that the embryonic buds of the ectodermal vesicle follow suit. The rebudding of the primary buds must be due in like manner to the establishment of two growing points in each primary bud. Such an explanation of polyembryony involves the whole problem of the physiology of budding, about which there is great diversity of opinion. According to Professor Child's theories of development and reproduction, any part of a system which, through a lowering of the rate of metabolism of

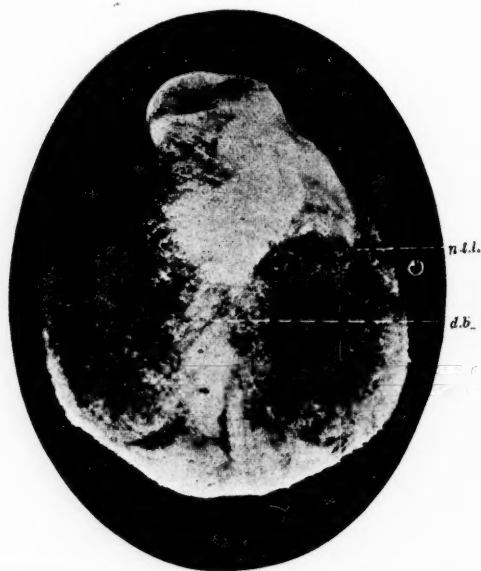


FIG. 6. Photograph, about one half natural size, of an embryonic vesicle just before birth, showing the two lateral placental areas, attached to the right and left sides of the maternal uterus, separated from each other by an area practically free of villi. The outlines of the four fetuses may be seen through the transparent, non-villous areas of the common chorionic vesicle.

the controlling part of the system, say the animal pole of the blastodermic vesicle, is liable to physiological isolation of parts at certain distances from the dominant region. When such isolation of parts occurs new centers of control arise, which produce buds capable of establishing whole new systems like the original. Thus in the particular case under discussion the rate of metabolism of the whole vesicle is lowered by parasitism to such an extent that the dominant growth center of the system no longer is able to hold the various subsidiary growth regions under control, and new centers of control arise at points determined by secondary pressures exercised by the uterine grooves, as explained above. Further complexities in development are of the nature of adjustments of four separate fetuses compelled to carry on growth and differentiation within a common chorion which had already been established before physiological isolation of the four embryonic rudiments had taken place. According to current theories, reproduction is a result of senescence and, on this basis, it may be assumed that the young blastodermic vesicle, weakened by the ravages of parasites, is precociously old, and therefore tends to reproduce by a process of dichotomous budding. Later, when the parasite completes its active period and goes into encystment, and when the embryos begin to gain new vigor through the absorption of the maternal nutrient fluids, general rejuvenation occurs, the rate of metabolism increases, so that no further isolation of parts occurs. In this connection it is of interest to note that in the *mulita* armadillo of the Argentine budding goes one or two steps further than in our species and from eight to twelve fetuses result. The writer recognizes the extremely hypothetical character of the explanation of polyembryony here offered and would welcome any suggestion that would lead to a more satisfactory theory. It would be of interest, however, to know whether there is an egg parasite in the *mulita*, and the writer intends to test this possibility in the near future. If

this should prove to be the case the hypothesis here offered would receive a striking support. A detailed description of the life history of the parasite here discussed is in preparation and will no doubt soon appear in print.

THE PROBLEM OF THE ORIENTATION OF THE COMPOUND
VESICLE IN THE UTERUS AND THE ORIGIN OF PAIRS

One of the most striking facts that came to light in the early stages of the present studies is that the vesicle is distinctly a bilateral object and that this bilaterality is strictly in accord with the bilaterality of the uterus. It was noted that one pair of fetuses was attached to the right and the other to the left placental disc. It was furthermore discovered that this pairing is not merely a mechanical adjustment of the fetuses to the shape of the uterus, but involves resemblances in stage of development, size and the minutiae of inherited peculiarities. To explain this condition we offered the conjecture that each



FIG. 7. Photograph of a vesicle a little younger than that shown in Fig. 6, split open along the mid-ventral line, showing the umbilical attachments of the quadruplets.

pair is derived from one of the first two cleavage blastomeres, an idea borrowed from the literature on human duplicate twins. Such a theory, however, involves the difficulty of explaining how the cell descendants of one blastomere would come to occupy a position with reference to one or the other lateral halves of the uterus. The axial orientation of the vesicle is determined by the fact that it always becomes attached by an area of trophoblast at the animal pole, but there is no mechanism for preserving a bilateral orientation. There is on the other hand good evidence, as brought out in the last section of this paper, that the definitive bilaterality of the compound embryonic vesicle is imposed upon it by certain definite bilateral conditions within the uterus, which result in the vesicle being pressed dorso-ventrally and elongated laterally so as to acquire a bilaterality in conformity with that of the uterus. Thus bilaterality and pairing of fetuses are strictly secondary results and bear no relation to any axes of the egg or planes of cleavage. The closer resemblances between the individuals of pairs and their closer placental association are due to their common origin from one primary bud, which means that they are genetically more closely related than are the members of opposite pairs. Mirrored-image effects are also made more intelligible by our knowledge of the mode of origin of a pair from a single primary bud, in that when an inherited peculiarity on the right margin of one individual of a pair is found on the left margin of its partner, it means that some median primordium of the primary bud has been split by the secondary budding, so that the resultant character is found repeated on the adjacent sides of the two fetuses. Dichotomies of primordia of this sort also serve to explain the distribution of many peculiarities inherited by the quadruplets from the parents.

SEX RATIOS AND SEX DETERMINATION

In a collection of 182 sets of fetuses sufficiently advanced to determine with certainty the sex there has appeared no exception to the rule that all fetuses in a

set or litter, whether the number of individuals in a set be 2, 3, 4 or 5, are of the same sex. Of these 182 sets 88 were female and 94 male, which would seem to indicate that the two sexes are about equal in numbers. A total of 210 sets have come under the writer's observation, and of these four showed 5 fetuses, four showed 3 normal and 1 degenerate individuals, and in one case twins were born, due probably to the degeneration of a pair of fetuses. There are no authentic cases of less than four embryos being produced, but there are four cases, or less than 3 per cent. of the total number, in which there is exhibited a tendency toward an increase in the normal or typical quadruplet condition. This may be a progressive tendency and might conceivably result in numbers of fetuses resembling those produced by the mulita. The fact that the individuals of a polyembryonic litter are invariably of the same sex supports certain current views regarding the problem of sex determination. In particular it shows clearly that sex must be determined prior to the separation of the embryonic materials from which the four fetuses arise. Since, from the standpoint of cell lineage, this separation must take place at least as early as the cleavage stages, it would appear practically certain that sex is predetermined in the undivided oosperm. It has been claimed that the data with reference to sex in the armadillo might as readily be used as evidence of the control of sex by environment; for it is claimed that the environment of the four fetuses in a common chorion is as nearly identical as it could be made under controlled conditions. I claim, however, that there is no greater environmental uniformity here than exists in cases where several fetuses develop simultaneously in a single uterus. In both cases the individual fetuses have separate amnia, separate placentæ and unmixed fetal blood. The enclosure within a common chorion is a matter of secondary importance since each fetus is isolated completely in the really important ways just mentioned. Moreover it is certain that pronounced differences in nutrition and

rate of development frequently occur, as is evidenced by the facts that one pair of fetuses is often strikingly larger than the other. If sex is capable of being altered by nutritive factors one would expect to note some differences of sex within a set in which some fetuses have evidently had a much less favorable developmental environment than others. There are in my collection several sets of quadruplets in which one pair of fetuses is very decidedly larger and more advanced than the other. A condition of this sort is probably to be traced back to a very early period, as early as that shown in Fig. 5, where it is readily seen that one pair is distinctly in advance of the other. Patterson has also stated that it is not uncommon to find one of the primary bud primordia dividing in advance of the other. If sex is capable of being influenced by metabolic inequalities of any sort, there should be opportunity here for the operation of such influence. Yet there is not a single instance in which there is any diversity of sex within a set of fetuses derived from a single germ cell.

Cytological studies of the germ cells are in strict accord with current chromosomal hypotheses of sex determination. The female diploid number of chromosomes is 32 and the haploid 16; the male diploid 31, producing two kinds of spermatozoa, one with 15 and the other with 16 chromosomes. There occurs in the reduction division an odd chromosome like that described for other vertebrates, notably the birds and man as shown by Guyer. The presumption is that this odd chromosome plays the same rôle in the determination of sex as it is assumed to play in an extensive array of animals. The character of the evidence is the same in all cases. On this basis it may be claimed that in the armadillo an egg fertilized by the 15 chromosome type of spermatozoon produces a male and one fertilized by a 16 chromosome type, a female. Environmental factors are powerless to alter the sex thus determined.

ANALYSIS OF PREDETERMINATIVE VERSUS EPIGENETIC
FACTORS IN DEVELOPMENT

According to the proponents of the pure line hypothesis the genotypic constitution of an individual is fixed at the time of fertilization of the ovum. On this assumption the fertilized egg of the armadillo has a fixed and definite hereditary potentiality and, unless inequalities of some sort are introduced during development, *i. e.*, epigenetically, the four fetuses should be identical. The degree of difference then that actually exists among the individuals of a given set of quadruplets should be a measure of the potency of the epigenetic factors of all kinds, while the degree of correlation among the individuals of a set should serve as a criterion of the relative strength of the predetermining factors. It has been customary to employ the data derived from comparisons of human duplicate or identical twins as a measure of the extent of predetermination, but such data are unreliable for two reasons. It is impossible, on the one hand, to be sure whether or not such twins are the product of one egg, and in practically all cases the measurements and comparisons are made comparatively late, so that the two individuals may have had a divergent environmental experience. In both of these respects the armadillo quadruplets offer superior advantages and should in the future take the place of human twins as material illustrating the potency of predeterminative factors in development, for not only do we know for certain that each set of quadruplets is the product of a single egg, but the amount of material is thoroughly adequate for statistical treatment, and the individuals are compared before birth, so that their pairing and placental relations are known. Coefficients of polyembryonic correlation have been determined for a very large number of characters, such as the numbers of plates or scutes in the various regions of the armor, and these have ranged from 0.92 to 0.98. These coefficients are strictly of the order of those determined for antimerically paired structures of the same indi-

vidual. In other words, these quadruplets resemble one another as closely as do the right and left sides of single individuals. One might readily make the claim that the quadruplets are simply four parts of one individual, since they have been derived by a process of asexual budding from a single embryonic vesicle. The closest of ordinary blood relations have coefficients of correlation of a decidedly lower order, that of brothers being about 0.5; hence the polyembryonic relation is much closer than a mere fraternal one. We may conclude then that the predetermining mechanism works accurately up to from 91 to 98 per cent. and that epigenetic disturbances or inequalities effect alterations in the end result ranging from 2 to 8 per cent. One of the most fundamental postulates of the sciences of taxonomy and phylogeny is to the effect that degrees of resemblance are criteria of blood relationship. This postulate is strongly supported by the facts just given, since the closest resemblances ever found to exist between individuals are here the result of the closest possible blood relationship; for no closer genetic relationship could well be conceived than that involved in the known origin of these armadillo quadruplets.

A subsidiary question arises as to what kind of epigenetic factors operate in inducing dissimilarity among the polyembryonic offspring of a set. Studies of the heredity of certain characters and of the distribution of certain units among the quadruplets lead to the conclusion that the most important differences are due to imperfections in the mechanism for distributing germinal materials, a mechanism which has visible expression in the mitotic complex of cleavage. It seems probable that certain materials which condition the development of certain characters are not distributed with exact equality to the first two blastomeres. This would make one half of the embryonic vesicle different in potentiality from the other. A similar inequality might exist in the second cleavage and in subsequent cleavages. Mere differences

in nutriment, position, etc., are impotent to influence any but dimensional characters, such as length, weight and relative position of units. Inherited characters are affected only by changes in the germinal materials, and such changes might readily be due, as indicated, to inequalities in the distribution of material particles during cleavage.

MODES OF INHERITANCE IN POLYEMBRYONIC OFFSPRING

The material for the study of inheritance consists of nearly two hundred sets of quadruplets and the armor of the mothers. Without breeding in confinement, which is not at present practicable, no data concerning paternal inheritance are available. Since, however, there is no sex dimorphism with regard to the characters studied, and since males and females inherit alike from the mothers, one can discover all the essential laws of inheritance governing the polyembryonic relationship from a comparison of individuals in sets and of quadruplets with their mothers. After an exhaustive study of this large mass of material the chief general laws discovered are to the effect that single meristic variates, such as particular scutes, and also aggregates of these elements, as for example the total numbers of these units in a given region of the armor, are inherited in the alternative fashion and show only a minor degree of blending. This is an unexpected result in view of the fact that it has been the general impression that meristic variations usually exhibit blended inheritance and substantive variations obey the laws of Mendelian inheritance. In this material it has been found that single scutes, recognizable through some marked peculiarity, such as a tendency to split or to fuse with a neighboring element, is inherited as a Mendelian dominant character. If the mother has the character unilaterally or in one band of the armor, one or more of the offspring invariably exhibit the character either unilaterally or bilaterally, either in one band or reduplicated in two or more bands. Again a single scute

peculiarity in the mother may be inherited by one, two or all of the offspring, as a row of peculiar scutes starting at the place where the one peculiar element occurs in the mother. Such fluctuations in the expression of a type peculiarity may conceivably be due to epigenetic factors, and suggest duplication of factors of the neo-mendelian sort.

One of the problems of this material is to determine why one individual or one pair inherits a dominant peculiarity from the mother, while the others do not. They all have the same germinal constitution at the beginning and that some should inherit the character and others not seems to imply that there must have occurred a segregation of maternal and paternal inheritance factors during cleavage. The distribution of the characters so as to produce mirrored image effects, together with this segregation of parental characters, seems to imply a sort of dichotomous distribution of some material basis that conditions the development of the characters so segregated and distributed. Such determiners need not be conceived of as Weismannian elements, but that they have corporeal existence appears to the writer as an unavoidable conclusion.

The data upon which these conclusions are based are of highly complex character and have not yet been published in extenso. The demonstration of the tenability of the conclusions can be made only by the use of much more illustrative material than can be presented in a paper of this sort. In conclusion it may be said that, although the inheritance phenomena have occupied more time and attention than any other phases of the armadillo work, the conclusions reached are less precise and less satisfactory than those in other fields. Yet it is imperative that we should find out just what new light this unique material and unparalleled genetic situation may be able to throw upon the general problems of inheritance. The detailed data and conclusions regarding these intricate problems are being elaborated for publication in the near future.

DARWINISM IN FORESTRY

RAPHAEL ZON

U. S. FOREST SERVICE

THE centennial anniversary of the birth of Charles Darwin was the occasion for many interesting reviews of what Darwinism has done for the biological sciences. In all these reviews, however, scarcely any reference is made to forestry. Yet historically and inherently there is a most remarkable and unique connection between Darwinism and forestry.

On April 10, 1860, soon after the appearance of the first edition of the "Origin of Species," Darwin wrote to his friend C. Lyell:

Now for a curious thing about my book, and then I have done. In last Saturday's *Gardeners' Chronicle*, a Mr. Patrick Matthew publishes a long extract from his work on "Naval Timber and Arboriculture," published in 1831, in which he briefly but completely anticipates the theory of Natural Selection. I have ordered the book, as some few passages are rather obscure, but it is certainly, I think, a complete but not developed anticipation! One may be excused in not having discovered the fact in a work on Naval Timber.¹

And three days later, on April 13, 1860, he wrote to J. D. Hooker.²

My dear Hooker—Questions of priority so often lead to odious quarrels, that I should esteem it a great favor if you would read the enclosed. If you think it proper that I should send it (and of this there can hardly be any question), and if you think it full and ample enough, please alter the date to the day on which you post it, and let that be soon. The case in the *Gardeners' Chronicle* seems a little stronger than in Mr. Matthew's book, for the passages are therein scattered in three places; but it would be mere hair-splitting to notice that. If you object to my letter, please return it; but I do not expect that you will, but I thought that you would not object to run your eye over it.

¹"The Life and Letters of Charles Darwin," by F. Darwin, 1898, New York, Appleton & Co., p. 95.

²*Ibid.*, pp. 95 and 96.

The statement to which Darwin referred in his letter to Hooker appeared in the *Gardeners' Chronicle* on April 21, 1860 (page 362), and is this:

I have been much interested by Mr. Patrick Matthew's communication in the number of your paper dated April 7th. *I freely acknowledge that Mr. Matthew has anticipated by many years the explanation which I have offered of the origin of species, under the name of natural selection.* I think that no one will feel surprised that neither I, nor apparently any other naturalist, *had heard of Mr. Matthew's views,* considering how briefly they are given, and that they appeared in the *appendix to a work on Naval Timber and Arboriculture.* I can do no more than offer my apologies to Mr. Matthew for my entire ignorance of this publication. If another edition of my work is called for, I will insert to the foregoing effect.³

In the Historical Sketch⁴ which he added to the later editions of his book Darwin gives Matthew credit for the Nature's law of selection in the following words:

In 1831 Mr. Patrick Matthew published his work on "Naval Timber and Arboriculture," in which he gives precisely the same view on the origin of species as that (presently to be alluded to) propounded by Mr. Wallace and myself in the *Linnean Journal*, and as that enlarged in the present volume. Unfortunately, the view was given by Mr. Matthew very briefly in scattered passages in an Appendix to a work on a different subject, so that it remained unnoticed until Mr. Matthew himself drew attention to it in the *Gardeners' Chronicle*, on April 7th, 1860. The differences of Mr. Matthew's view from mine are *not of much importance*: he seems to consider that the world was nearly depopulated at successive periods, and then re-stocked; and he gives as an alternative, that new forms may be generated "without the presence of any mould or germ of former aggregates." I am not sure that I understand some passages; but it seems that he attributes much influence to the direct action of the conditions of life. *He clearly saw, however, the full force of the principle of natural selection.*⁵

In a letter written by Darwin to J. L. A. de Quatrefages on April 25, 1861, he referred to Patrick Matthew's explanation in a postscript as follows:

I have lately read M. Naudin's paper, but it does not seem to me to anticipate me, as he does not show how selection could be applied under

³ *Ibid.*

⁴ "The Origin of Species," 1878, p. xvi—Historical Sketch.

⁵ *Ibid.*

nature; but an obscure writer on forest trees, in 1830, in Scotland, most expressly and clearly anticipated my views—though he put the case so briefly that no single person ever noticed the scattered passages in his book.

Grant Allen in his biography of Darwin (1888) calls Patrick Matthew the unconscious author of the principle of natural selection which he applied in his book on naval timber to the entire Nature.

Here then is a most interesting fact which seems to me of deep significance to foresters. The first Darwinian, who twenty-nine years before Darwin formulated the law of natural selection, was a forester. I shall not attempt here to compare Darwin's and Matthew's views on natural selection. Matthew's book, the full title of which is "Naval Timber and Arboriculture, With Critical Notes on Authors Who Have Recently Treated the Subject of Planting," is accessible in the Congressional Library. The chapter on Nature's Law of Selection I hope can be reprinted in the next issue of the *Proceedings of the Society of American Foresters*, so that every one will be able to draw the comparison for himself.

In bringing together this evidence I am very far indeed from any desire to detract in the least from the great service which Darwin rendered to science. It was Darwin who first gave flesh and blood to the idea of natural selection. It was his wonderful interpretation of all biological facts in the light of natural selection that made the latter the universal law applicable to the entire organic world. Before this accomplishment the claims of all others must sink into obscurity.

My purpose in assembling these records is twofold: *First*, to restore the memory of one who ploughed the same fields as we do now, the name of a forester whose idea, although it did not perish, slumbered almost unknown for nearly thirty years until another and bigger man brought it to life and general recognition; and *second*, to offer an explanation of the reason why a forester above all others should be the one to observe and formu-

late the law of the struggle for existence as the basis for natural selection and the origin of new species.

My first purpose, I hope, has been accomplished by quoting extracts from Darwin's correspondence. The second still remains.

There is nothing accidental, in my opinion, in the fact that a forester should be the first to observe the struggle for existence and its bearing upon the development of the new varieties, because there is no other plant society in the world which presents a more striking example of the struggle for existence and of natural selection than the forest. Nowhere else, also, can the law of this process be more fully studied.

The regular decrease in the number of trees on a given area with increase in age forms one of the earliest observations of the foresters, who, at a time antedating Darwin, properly gave this process the name of the struggle for existence, the struggle for the necessary growing space. The foresters have discovered the laws governing this process, a process in which almost 95 per cent. of all trees that start life in the stand perish, and in the form of yield tables have expressed it quantitatively, have measured and weighed it. They have shown how this struggle for existence varies with the species, climate, drainage and soil conditions, and age of the stand; that it is more intense, and consequently the differentiation into dominant and suppressed classes occurs earlier with light-needing species than with shade-enduring ones. In a climate most suitable to the species and on favorable situations this struggle again results in more rapid differentiation into dominant and suppressed trees than when the species grow outside of their optimum range and on poor soils. These are elementary and fundamental facts known to foresters for many years.

The foresters have not only observed these facts, but they have also furnished an explanation for them. The more favorable the conditions of growth, the greater is the development of the individual trees; the earlier,

therefore, begins the struggle for space and the differentiation into dominant and suppressed, with the subsequent dying out of the latter. They have followed this process throughout the entire life of the stand, have established its various degrees of severity, and have discovered its culmination during the period of the most rapid growth in height. This struggle for space and light is the basis of the forester's operations, as only by utilizing and controlling it is he capable of producing wood of high technical qualities, tall cylindrical boles, free of branches, and wood with uniform annual rings possessing great elasticity. Without this struggle there is no forest, there is no production of valuable timber, save firewood.

The struggle for existence in a forest stand is not confined to individual members of the same age or the same story, but the forest, as a whole, battles for its existence against the adjoining meadow, swamp or shrub vegetation; the old trees against the young growth that comes up under them; groups of trees of different species or of different ages against each other. In this struggle the forest accomplishes what no other vegetation does; namely, it actually changes the climate over the area occupied by it, and makes it inhospitable for its enemies. The forest creates its own interior environment to which its own members are completely adapted, but in which other species find either too much or too little light, the humus too scant or too deep, or too acid, the temperature too high or too low. Whatever it may be, the forest's competitors are eliminated through the changed environment. To change this environment, however, there must be a close stand, there must be present the struggle for existence among the individual members of the stand. Through interior struggle among its own members the stand secures resistance against invasion by other vegetation. How manifoldly broad and deep, then, is the struggle for existence in the forest.

When we come now to natural selection nowhere else is

it expressed in such fullness and so strikingly as in the forest. The forest is a natural breeding place in which constantly only the trees best adapted to the climate and the situation are allowed to remain. In the forest only the conquerors in the struggle for existence are the ones which produce seed in abundance. During a seed year the dominant and co-dominant trees produce seed in large quantities; the intermediate trees, which may properly be called the candidates for suppression, participate but little, and then only in exceptionally good seed years, while the oppressed and suppressed do not bear seed at all. With what rigidity, then, must the natural selection go on in a forest, if we consider *first* what a small percentage of trees in a stand of the same generation come to be conquerors in the struggle for existence; *second*, the great age reached by trees; *third*, the numerous generations of trees that have succeeded each other in the same forest; and *fourth*, the relatively limited capacity of tree seeds for dissemination. With each generation the forest trees must become more and more delicately adjusted and adapted to the given conditions of growth. The new generation inevitably arises from seed sown by the best developed trees, from those which have withstood the long and intense battle not only against Nature alone, but against Nature in the presence of competitors. Of this possibly only 1 per cent. or less will reach maturity and be able to continue the species. No wonder, therefore, that in spite of search for new species all over the world so few forest trees have been successfully introduced into new countries and so little progress has been made with the artificial improvement of them. So perfect is the natural selection in the forest, so fine is the adjustment between the environment and the forest trees, that it is almost impossible for man to approach it. I do not mean the introduction of trees for park purposes or breeding new varieties for some other purpose than timber; I have in mind only the establishment of natural forests and the production of timber.

The natural selection forms also the basis of the forester's operation in selecting trees for seeding purposes, in making regeneration cuttings, in collecting seed for reforestation and so on.

These few facts are enough to show with what fullness and force the principles advanced by Darwin are expressed in the forest. If agriculture furnished Darwin with many examples of artificial selection upon which he built by analogy his principle of natural selection, the forest, of all plant formations, furnishes the most striking examples and proof of the latter. *As a matter of fact, forestry as an art is nothing else but the controlling and regulating of the struggle for existence for the practical ends of man; forestry as a science is nothing else but the study of the laws which govern the struggle for existence.*

Is there anything strange, therefore, that it was a forester who first formulated the principles of natural selection? Is there anything strange, also, in the fact that it was also foresters who have laid the foundation for what has come to be known as ecology, which is the logical development of Darwinism? Because of the fact that the forest is the highest expression of plant life, the foresters occupy the strategic position from which they command vistas accessible only with difficulty to other naturalists. In this lies the strength of forestry, its peculiar beauty, and the debt which science owes to it.

GENETICAL STUDIES ON *ÆNOTHERA*. IV

II

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3. HYBRIDS OF *grandiflora* *B* \times *biennis* *D* IN THE F_2 GENERATION

In my last paper (Davis, '12a, pp. 392-406) there was described an especially interesting F_1 generation, culture 11.35, of the cross *grandiflora* *B* \times *biennis* *D*. The *biennis* male parent of this cross (Davis, '12a, pp. 385-389, Figs. 1-3) was of a race with the stem coloration characteristic of *Lamarckiana*, i. e., the papillæ or glands at the base of long hairs were colored red on green portions of the stem. The *grandiflora* female parent bears the same type of papillæ, but they follow the color of the stem and therefore lack the red over green portions of the stem. The *biennis* parent then presented a character, the red coloration of the papillæ, that might be expected to be present or absent in the F_1 , and to present an alternative inheritance in the F_2 generation.

It will be remembered that in the F_1 generation of this cross, *grandiflora* *B* \times *biennis* *D*, consisting of 180 plants, two sharply contrasted classes appeared (Davis, '12a, p. 395). Class I was represented by 12 plants which had the stem coloration of the *biennis* parent (red papillæ on green portions of the stem). Class II was represented by 168 plants with the stem coloration of the *grandiflora* parent (stems above clear green). Other peculiarities of these classes are described in the paper cited above, but we are concerned at this time chiefly with the behavior of this color character. The mixed conditions in this F_1 generation naturally suggested the probability that the male *biennis* parent was heterozygous with respect to the red coloration of the papillate glands and that it formed

two classes of gametes with and without the factor responsible for this character.

Although it is true that the form *biennis* *D* in later generations has been uniform as to the stem coloration described above it by no means follows that the original plant of 1910, which furnished the gametes of the cross, was homozygous for this character. I have already noted the fact (Davis, '12a, p. 386) that types occur wild similar to *biennis* *D* except for their clear green stems. Consequently the original plant may have been heterozygous with respect to factors for red papillæ and in my later generations I may have isolated a homozygous line.

Last summer I grew the reciprocal of the cross described above, *i. e.*, a cross *biennis* *D* \times *grandiflora* *B* which involved the same parent plants as in the first. The F_1 generation of 103 plants, culture 12.11, was brought to maturity and consisted of the same two clearly defined classes. Class I, consisting of 87 plants, presented the stem coloration of the *biennis* parent (red papillæ on green portions of the stem). Class II, consisting of 16 plants, presented the stem coloration of the *grandiflora* parent (stem above clear green). There was a disproportion of the numbers as in the previous case, but in the reciprocal cross the plants with red papillæ were in a large majority, 87:16, instead of being in a small minority, 12:168, present in the first cross. Other peculiarities of these classes were the same as in the first cross. Again the mixed conditions in the F_1 reciprocal cross suggested the probability that the *biennis* parent, in this case female, was heterozygous with respect to the red coloration of the papillate glands and that it also formed two classes of gametes with and without the factor responsible for this character.

The two classes of hybrids in the F_1 generation described above appear to present a phenomenon similar to the "twin hybrids" of De Vries ('07) which result when *Œ. biennis* or *Œ. muricata* are pollinated by *Lamarckiana* or by one of its derivatives (*e. g.*, *rubrinervis*, *brevistylis*

or *nanella*). Critics have pointed out that this behavior indicates that *Lamarckiana* is heterozygous or hybrid in character since it must form at least two different types of male gametes. De Vries apparently believes that the "twin hybrids" in my crosses show that the *grandiflora* parent is in a condition similar to that of *Lamarckiana* and that the "twin hybrids" are due to the mutations of *grandiflora*. My interpretation of the behavior is quite the opposite, for, as will be shown, the evidence indicates that the *biennis* parent, with respect to the characters concerned, is heterozygous and that the race of *grandiflora* is stable. If this is true the evidence does not indicate that the race *grandiflora B* exhibits with respect to these characters the habit of mutation as claimed by De Vries ('12, p. 30).

Among some 300 plants of *grandiflora* grown from wild seed and 200 more grown in isolated lines none have presented red-colored papillæ over green portions of the stem. All green-stemmed forms of *biennis* have proved perfectly true to this character. One of the best known types of green-stemmed *biennis* is the Dutch plant extensively grown by De Vries and Stomps, and this, as far as I know, is constant. Furthermore, all green-stemmed F_1 hybrids have in later generations proved constant to this form of coloration. There is thus much evidence that the absence of red in papillæ over green portions of the stem constitutes a homozygous condition. The type *biennis D*, as stated before, can not be distinguished in other respects from wild plants which lack the red coloration in their papillæ, and it seems probable that this assemblage is a mixed population in which some plants are heterozygous with respect to the character of their stem coloration.

Although I can not as yet present experimental proof that the red coloration of papillæ is a character dominant to its absence, we should expect this to be the case because anthocyan coloration is obviously a character in addition to that of the green and because its inheritance appears

to be alternative. It is on this hypothesis that I shall treat the red coloration of papillæ as a dominant character when for convenience employing a Mendelian notation in the accounts that follow.

It became a matter of interest to determine how representatives of Class I and Class II would behave in the F_2 when selfed and how they would behave when crossed reciprocally. Therefore I selected a plant, 11.35 m (Davis, '12a, Figs. 6 and 7), as representative of Class I, and a plant, 11.35 a (Davis, '12a, Figs. 5, 8 and 9), as representative of Class II, and according to my plan (Davis, '12a, p. 399) carried these in pure lines into an F_2 and also grew the crosses 11.35 $m \times a$ and 11.35 $a \times m$. Furthermore, a large F_2 generation was grown from an especially interesting plant 11.35 La (Davis, '12a, Figs. 10, 11, 12 and 13), also representative of Class II, which resembled *Oenothera Lamarckiana* closely in certain particulars. These cultures will now be briefly described.

1. *The F_2 Generation from 11.35 m .*—From this plant, with red papillæ on green portions of the stem as in the parent *biennis*, the contents of one capsule, 413 seeds, were sown. The culture, 12.43, produced 180 seedlings, of which 166 plants were brought to maturity. Among these, 86 plants presented the stem coloration of 11.35 m and the *biennis* parent of the cross, and 80 plants presented the stem coloration of the *grandiflora* parent.

Let us assume the formula for the *biennis* parent to be Rr (R standing for the presence of the factor responsible for the red color of the glands and r for its absence); i. e., the *biennis* parent is held to be heterozygous for this character and to form two classes of gametes, viz., R and r . Let us assume that the formula for the *grandiflora* parent with respect to this character is rr . The F_1 hybrid plant 11.35 m would then be expected to have the formula Rr and to produce gametes R and r . These gametes in chance combinations should give F_2 hybrids in the proportions $1RR:2Rr:1rr$, which would be a 3:1 ratio with respect to the appearance of the character R (red

papillæ). I lay no stress on the fact that in my small cultures the numbers were 86R:80r, but merely wish to note the point that in this F_2 generation two classes appeared sharply distinguished by the presence or absence of the character under discussion.

I was unable to differentiate in this F_2 other characters on the plants 11.35m and 11.35a associated with the presence or absence of the red papillæ (see Davis, '12a, p. 395). There was a wide variation in habit, leaf, form, inflorescence, flower proportions and flower size (petals 2.2-3.9 cm. long), a variation that seemed unrelated to the presence or absence of red papillæ. In this culture also appeared a group of 15 dwarfs, recognizable when young rosettes, which at maturity were from 5-6 dm. high, sparsely branched, and with a foliage of narrow leaves; 6 of these dwarfs had the stem coloration of the *biennis* parent (red papillæ) and 9 that of *grandiflora*.

Among the plants with red papillæ on the stems I selected an individual, 12.43g, which among my hybrids with the stem coloration of *Lamarckiana* most resembled that form. I shall make this plant the starting point of a pure line with the hope that in later generations I may find variants still closer to the *Lamarckiana* type which may be isolated by selection. Whether the plant is homozygous with respect to the red coloration of the papillate glands is a point to be determined by the next generation.

2. *The F_2 Generation from 11.35a.*—From this plant with the stem coloration of the *grandiflora* parent (papillæ green over green portions of the stem), the contents of one capsule, 432 seeds, were sown. The culture, 12.42, produced 165 seedlings of which 147 plants were brought to maturity. These presented uniformly the stem coloration of the F_1 hybrid plant 11.35a and of the *grandiflora* parent. It seems then safe to conclude that such a plant as 11.35a is homozygous as to its stem coloration with possibly the formula of a recessive (*rr*) lacking the factor that produces the red color in the papillate glands. This position is supported by the evidence from the much

larger F_2 generation grown from the sister plant of the same Class II, 11.35*La*, where 532 plants agreed in having this same type of stem coloration characteristic of *grandiflora*.

The culture was remarkable for the length and breadth of its leaves, which far surpassed that of the parents of the cross and for its general vigor. In these respects there was marked progressive evolution. The flower size, however, was below the *grandiflora* type, the petals ranging from 1.5 to 2.8 cm. long (those of *grandiflora* being about 3.3 cm. long). Since none of these plants appeared to present the possibility of developing the stem coloration of *Lamarckiana*, I have not considered it worth while to follow the family further.

3. *The Cross 11.35 m × a and its Reciprocal 11.35 a × m.*—These crosses were made to determine whether or not the peculiarity of the red glands with the other correlated characters was in any sense or degree sex-limited. Thus if these characters were carried by the male gametes from the plant 11.35*m*, the progeny of the cross 11.35 *a × m* should have the peculiarities of Class I, while the progeny of the cross 11.35 *m × a* should have the peculiarities of Class II. A behavior of this general nature has been described by De Vries ('11) in his paper on double reciprocal crosses.

From the cross 11.35 *m × a* the contents of one capsule, 276 seeds, were sown. The culture, 12.45, gave 143 plants which were brought to maturity. Of these, 50 plants presented the red-colored papillæ characteristic of 11.35*m* and of the *biennis* parent, and 83 had the coloration of 11.35*a* and of the *grandiflora* parent. On the hypothesis developed through the cultures previously described the plant 11.35*m* should have the constitution *Rr* and the plant 11.35*a* should have the constitution *rr*. The female gametes of 11.35*m* should then have been of two sorts (*R* and *r*), the male gametes from 11.35*a* should have been all similar (*r*), and the plants of the culture distinguished as 50*Rr* and 83*rr*. The expected ratio of the two classes

would be 1:1, provided that the female gametes R and r were formed in equal numbers and mated in equal proportions with the male gametes (r). It is at least clear from this culture that the factor for red glands (R) is in this case carried by a certain proportion of the female gametes and that the female gametophytes for the plant 11.35 m must be of two sorts (R and r).

The plants of this culture, 12.45 (11.35 $m \times a$), failed to exhibit consistently the other differences associated with the presence or absence of red glands as illustrated by the two F_1 types 11.35 m and 11.35 a . There was a marked progressive advance over the parent species, *biennis* and *grandiflora*, in leaf size and general vigor, but not in flower size, the petals ranging from 1.5 to 3.2 cm. in length.

From the cross 11.35 $a \times m$ the contents of one capsule, 223 seeds, were sown. The culture, 12.44, gave 142 plants which were brought to maturity. Of these 23 plants presented the red-colored papillæ characteristic of 11.35 m and of the *biennis* parent, and 119 had the coloration of 11.35 a and the *grandiflora* parent. The proportions of these two types (23:119) is far from the expected ratio 1:1 on the hypothesis considered above, but it should be noted that the total number of plants in the culture (142) is small. The main consideration is, however, clear, viz., that the factor for the red papillæ is in this case carried by a certain proportion of the male gametes and that the male gametophytes from the plant 11.35 m must be of two sorts (R and r). Thus in both crosses (11.35 $m \times a$ and 11.35 $a \times m$) the character of the red papillæ is represented in certain of the gametes both male and female and the character is not sex-limited.

The plants of the culture 12.44 (11.35 $a \times m$) also failed to show consistently the other differences associated with the presence or absence in the F_1 of red papillæ. There was a similar progressive advance over the parent species in leaf size and vigor, and likewise no advance in flower size, the petals ranging from 1.3-3 cm. in length.

4. *The F₂ Generation from 11.35La.*—This plant, 11.35La (Davis, '12a, pp. 401–406, Figs. 10, 11, 12 and 13), was one of the most interesting of my hybrids because of its strong resemblance to *Lamarckiana* in buds and foliage. The coloration of the stem was, however, that of Class II, *i. e.*, it was *grandiflora*-like in the absence of red in the papillæ on green portions of the stem. I had no means of knowing, when this plant was selected as the parent of a second generation, that its type of stem coloration was probably recessive to that of the red papillæ as found on the *biennis* parent, and that I should be disappointed in my hope of obtaining in an F₂ some plants with the stem characters of *biennis D* and *Lamarckiana*. I now believe that such a form is unable to produce in later generations plants with red papillæ, and, since this is an important character of *Lamarckiana*, my efforts with this particular line of hybrids will be discontinued. The F₂ generation from this plant, however, from the genetical standpoint proved to be one of the most interesting that I have grown and well merits a brief description.

The contents of 14 capsules, containing 2,217 seeds, were sown, and after eight weeks gave a culture, 12.41, of 623 seedlings. An unusual mortality, apparently in a class of dwarfs, reduced the culture finally to 532 plants. The rosettes before they were half grown presented an extraordinary range of variation and it became possible to group them although this preliminary classification required considerable revision later. A large group of more than 100 rosettes presented broad closely clustered and crinkled leaves of the *Lamarckiana* type. Many of these rosettes when half grown were indeed indistinguishable from those of *Lamarckiana* at the same age. A smaller group of about 20 consisted of rosettes with narrow leaves; most of these developed into dwarf forms. Finally, the remainder, constituting what might be called the mass of the culture, contained rosettes ranging on the one hand from a number somewhat *grandiflora*-like to a few rosettes somewhat close to the *biennis* type, and be-

tween these extremes was an assemblage of intermediates impossible of classification. In short, this portion of the culture presented an excellent illustration of a relative segregation of characters, with the extremes, however, quite far from the pure parent types. As the culture grew to maturity a reclassification of the types became necessary and finally five groups were separated as described below.

Group *A* consisted of 132 plants which had the *Lamarckiana*-like foliage and short internodes (Fig. 16) of the parent F_1 hybrid 11.35*La*, together with the 4-angled buds and flower form of this plant. These plants developed from the group of rosettes with broad crinkled leaves of the *Lamarckiana* type. The size at maturity ranged from plants 1.3 m. high to dwarfs 4 dm. in height; the habit and leaf size exhibited great variation. The extreme types of dwarfs (13 in number) had very much the habit of *nanella*. The flowers varied greatly in size, petals 3.5–1 cm. long, with the stigma both above and below the level of the anthers. There was, therefore, in this group a decided segregation of flower size. A peculiar feature of these flowers was the very common cutting of the petals at the edge into narrow segments as in laciniate varieties of flowers. This is, as far as I know, a new character in the genus *Ænothera*. The greatest development of leaf size and extent of crinkling observed in this group is illustrated in Fig. 17, which shows two rosette leaves of one of the hybrids, 12.41*Lp*, compared with the rosette leaves of the parent types of *biennis* and *grandiflora*.

Group *B* contained 5 dwarfs, 3–4 dm. high, sparsely branched or not at all, and with narrow leaves. The buds and flowers were *grandiflora*-like in form, but the petals were only about 1.8 cm. long. These dwarfs were very delicate and presented the characters of *Ænothera* reduced in size to about the simplest terms. They recalled the class of dwarfs in the F_2 from the plant



FIG. 16. A type, 12.41Li, in the F_2 from the F_1 plant 11.35La, hybrid of *grandiflora* $B \times$ *bicennis* D, representative of Group A. A form similar to *Lamarckiana* in foliage, four-angled buds and fairly large flowers (petals 3 cm. long), but the stem coloration was the type of *grandiflora*, and the internodes were short as in *gigas*.

10.30Lb (Fig. 5), but were present in very much smaller proportions.

Group C comprised 7 plants having the habit of *grandiflora* with long branches from the base, but with narrow lanceolate leaves. The flowers were *grandiflora*-like (petals 3 cm. long), but the plants were not so high (about 8 dm.). The plants were distinguished with difficulty from certain forms in group F.

Group D consisted of 3 plants, short and very much

branched and with revolute leaves, very narrow above. The plants failed to flower.

Group *E* included 23 plants with a stiff upright habit and much-crinkled leaves. They resembled most closely the larger forms in group *A*, but were without the short internodes characteristic of those plants.

Group *F* contained the mass of the culture, 362 plants, after the separation of the groups described above. As a group it presented the best illustration of the relative segregation of characters that I have so far met in an F_2 generation. There was a very wide range of variation in flower size, habit and leaf form. A few types resembling *grandiflora* could be picked out at one end of the series, while at the other end were plants much closer to the *biennis* parent than have usually been found.

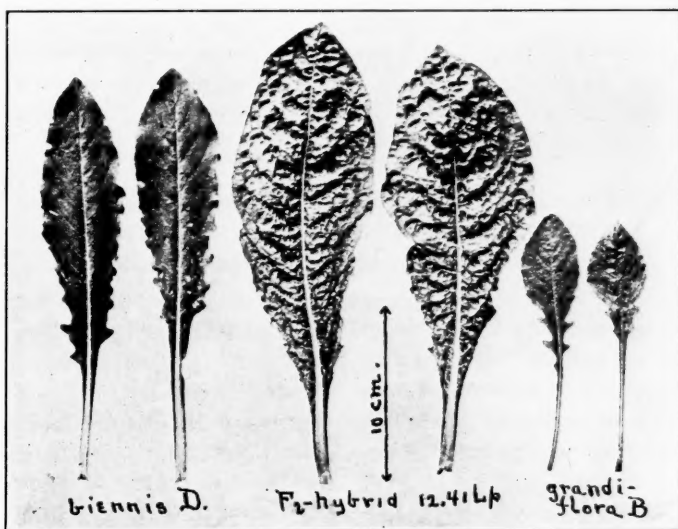


FIG. 17. Rosette leaves of a type, 12.41Lp in the F_2 from the F_1 plant 11.35La, hybrid of *grandiflora B* \times *biennis D*, representative of Group A. The rosette leaves are contrasted with those of the parents. The progressive advance in leaf size and crinkled texture is very striking. The dry weight of the two leaves of the hybrid (1.65 g.) was more than twice that of the two leaves of *biennis* and more than nine times that of the two leaves of *grandiflora*.

Curiously the tendency in this group appeared not to be progressive as to the size of flowers and other plant organs, but, instead, retrogressive. There were no plants with flowers larger than those of *grandiflora*, but in contrast a large number had flowers (petals 1-1.5 cm. long) much smaller than those of the *biennis* parent type (petals about 2 cm. long). The general tendency throughout this group, as well as that of group *A*, was distinctly downward as regards the size of the plant's organs. This is the first time that I have met with such a phenomenon in my observations on second generation hybrids of *Oenothera*.

Considering the culture, as a whole, it presented the same sort of extreme variation that has appeared in other F_2 generations. Many types were present which were taxonomically distinct from either parent of the cross and from the F_1 hybrid plant 11.35*La*. The groups of dwarfs included few individuals, but these were quite as puzzling in their extreme reduction in size as were the dwarf types described from the F_1 plants 10.30*La* and 10.30*Lb*.

4. A DISCUSSION OF THE BEHAVIOR OF THE HYBRIDS IN THE SECOND AND THIRD GENERATIONS WITH REFERENCE TO THE STABILITY OF MENDELIAN FACTORS

I wish briefly to point out what seem to me difficulties in interpreting the F_2 generations described in this paper in accordance with a strict Mendelian conception of the stability of factors. These difficulties are not presented as a criticism of Mendelism, for the data are not sufficient to justify conclusions, but it is well to note the problems.

As I understand the tenets of strict Mendelism it is assumed that the factors believed to be responsible for characters are stable. New characters are believed to appear either by the loss of factors or by their recombination in the gametes, with possibly the occasional introduction of new factors or modification of the old to give

"mutations." The process of segregation, of course, adds or subtracts nothing from the sum total of the factors but merely distributes them variously to the gametes that are formed. The increase or loss of factors in the offspring of a hybrid results from the mating of gametes which carry a greater or less number of factors.

Mendelism in its extreme expression may then be said to rest in large part on a law of *the conservation of factors*. This means that factors could never disappear from a genetic line of development if all of the gametes were mated and if all of the zygotes matured. It follows that the factors contained in an F_1 hybrid must all come out in an F_2 generation if that generation is sufficiently numerous.

The most striking specific problems brought forward by the data presented in this paper are:

1. The explanation of the large groups of dwarfs thrown off in the F_2 generations and repeated by certain plants in the F_3 .
2. The explanation of the well-defined progressive evolution, excluding the dwarfs, exhibited by these same cultures.

With respect to the dwarfs the ratio of their production in the most striking of the F_2 generations is as follows:

The F_1 hybrid 10.30*La* gave 141 dwarfs in a culture of 1,451 plants (ratio about 1:9).

The F_1 hybrid 10.30*Lb* gave 147 dwarfs in a culture of 992 plants (ratio about 1:5.7).

These are large ratios, considerably above the 1:15 which might be expected if the range of size depended upon so simple a matter as the presence or absence of two factors. It must be remembered that the dwarfs were very much smaller than either parent, as best shown in the dwarfs from the plant 10.30*Lb* (Fig. 5), where the proportion, about 1:5.7, was the largest. These small plants (Fig. 5), 3-4 dm. high, came from parents, *biennis* and *grandiflora*, about 10-15 dm. and 15-20 dm.

high, respectively. It is difficult to imagine fertile hybrids of such parentage much more reduced in their vegetative expression than are these dwarfs. Furthermore, the reduction was apparently a complete loss in the power of a greater growth, as was indicated by the dwarfs breeding true in the F_3 generation.

If the dwarfs were to be interpreted in so simple a manner as recessives from a cross where two factors for size were allelomorphic to their absence the ratio of the dwarfs to the mass of the culture should have been as 1:15. Why then in the mass of the culture, dwarfs excluded, was there no evidence of other classes? The two factors assumed must be of large value if their absence is to make the difference between the size of the dwarfs, 3-4 dm., and the size of the parents, an average of about 15 dm. There might be expected a class of giants to balance the class of dwarfs and in the ratio of 1:15. There should have been several other classes ranging between these giants and the dwarfs. With only two factors for size concerned, and these of such large value, it seems impossible that the fluctuating variations could conceal the presence of such classes. Yet the mass of the culture failed to exhibit them, and only the dwarfs could be separated as a class sufficiently distinct to warrant its designation. The mass of the culture ranged in size approximately between the limits of the parents; the gap between them and the dwarfs was not bridged by intermediates.

I am aware that the dwarfs might be explained as resulting from the presence of an inhibiting factor introduced into the cross, but again there should have been evidence of other size classes together with the dwarfs according as the inhibitor was present in a full or in a lessened amount or was entirely absent. These difficulties are in themselves of sufficient weight, let alone the general improbability of such a situation.

The explanation of the progressive evolution of an F_2 generation in which the culture with respect to cer-

tain characters appears to advance as a whole presents the second problem to be considered. This phenomenon was also best exhibited by the F_2 generations from the hybrids 10.30La and 10.30Lb. A large number of plants in these cultures bore flowers with petals 1 cm. longer than those of the *grandiflora* parent (petals about 3.3 cm. long), and the smallest flowers were, for the most part, two or more times larger than those of the *biennis* parent (petals about 1.3 cm. long); between these extremes was a very perfect range of intermediates. An explanation for the advance in flower size over that of *grandiflora* may, of course, be offered as a recombination of factors for large size on the hypothesis of multiple factors for the size of petals, but why was there not a balancing group of plants with flowers as small as or smaller than those of *biennis*? Even the dwarfs of these cultures had flowers larger than those of the *biennis* parent. The only plant having smaller petals was the extraordinary form 11.42j (Fig. 15). What had become in these cultures of the factors responsible for small size?

A similar situation was presented by the character of the foliage most markedly exhibited by the F_2 generations from 10.30La and 11.35a. The leaves throughout the mass of these cultures were much larger than those of the parents of the cross and much more crinkled. There was thus a marked progressive advance in leaf size with the absence of small-leaved classes of plants unless such were represented in the F_2 from 10.30La by the dwarfs. Admitting that possibility, the same problem must be faced as was discussed for the explanation of the dwarfs themselves which were present in a ratio of about 1:9, suggesting the 1:15 ratio with the presence of two factors for leaf size. Thus two factors for leaf size should give through the culture other classes besides those of the recessives, and these were not evident. Applying the hypothesis of multiple factors for leaf size one is compelled to enquire what has become of the fac-

tors or combination of factors that should give classes of small-leaved plants to balance the mass of the culture with its progressive advance in leaf size and degree of crinkling.

I present these problems not altogether as a criticism of the hypothesis of multiple factors which has been so ably applied in the recent *Oenothera* study of Heribert-Nilsson ('13), and by East, Hays and other investigators in various groups. This hypothesis has amply justified the confidence of its advocates, but it does not seem to me to be established as wholly satisfactory. There has been abundant evidence in my cultures of a segregation of size in the F_2 , but my question is whether this segregation may not be accompanied by a modification of factors whereby new sets wholly, or in part, take the place of the old. I do not think that East ('12) quite met the problem in his recent discussion of my data.

It has been suggested to me that the marked progressive advance in the size of organs in an F_2 generation may result from the continuance of the stimulus of heterozygosis (East and Hays, '12) apparent in the F_1 . Is it not, however, possible to view the phenomenon in the F_2 as the direct modification of the factors for size as a result of the cross? One of the most extreme illustrations that I have observed of such an advance is illustrated in Fig. 17, which shows rosette leaves of a certain F_2 hybrid plant (12.41*Lp*) in comparison with those of its parents. This plant failed to mature flowers and its study could not be continued. There was certainly indicated very profound changes in its vegetative organization.

Advocates of the hypothesis of multiple factors for size allelomorphic to their absence may claim the possibility of selective fertilization in the formation of zygotes preceding an F_2 or later generations. This possibility can not be disregarded, but we have no data for the *oenotheras*. There has been, however, in my experience usually a high degree of sterility in the seeds of *Oeno-*

thera hybrids following the F_1 for which no adequate explanation is known.

In one F_2 generation I have noted a distinct retrogression in the size of the flowers. This was the F_2 from the plant 11.35La briefly described in this paper. It contained no plants with flowers larger than those of *grandiflora* and a large proportion of the culture bore flowers as small as or smaller than the flowers of the *biennis* parent. The *biennis* parent in this cross was a rather large-flowered type (*biennis* D, petals about 2 cm. long) which made the retrogression appear the more marked.

A striking feature of the F_2 generations here considered has been the diverse progeny from F_1 sister plants of the same culture. Thus the F_1 hybrids 10.30La and 10.30Lb were sisters of the cross *grandiflora* B \times *biennis* A and the F_1 hybrids 11.35m, 11.35a and 11.35La were sister plants of the cross *grandiflora* B \times *biennis* D. Each plant gave its own peculiar set of types in the F_2 with peculiarities so pronounced that the blood relationship was much obscured. This is difficult to understand except on the theory that the parent stock was heterozygous; yet there has appeared no evidence of this in the cultures of the pure species. It is, however, clear that I have been working with complex material and it is not certain that the species of *Ænothera* employed in my crosses have been homozygous to the degree demanded for experimentation on the behavior of unit factors. For this reason I have endeavored to discuss the problems with full caution and I hold my point of view tentatively.

5. THE HABIT OF "MUTATION" IN *Ænothera Lamarckiana* DE VRIES CONSIDERED WITH REFERENCE TO THE
BEHAVIOR OF THE HYBRIDS BETWEEN
biennis AND *grandiflora*

Perhaps the most important observations on these hybrids of *grandiflora* and *biennis* in the second and third generations have been those showing a close parallelism

of their behavior to that of *Oenothera Lamarckiana*. Thus the hybrids have thrown off marked variants of new specific rank as does *Lamarckiana*. Certain of these new forms have held true and others have continued to throw variants as do some of *Lamarckiana*'s "mutants." One form (12.56*x*) appeared with a marked increase over the normal chromosome number (14) and apparently corresponds closely to the triploid "mutants" from *Lamarckiana* or its derivatives (Lutz, '12; Stomps, '12*a*). A most striking feature has been the production in successive generations of classes of dwarfs, plants which contrast sharply with the mass of the culture and which are stable.

This behavior of the hybrids appears to me to be of quite the same character as the "mutations" of *Lamarckiana*, but the results, here concerned with crosses between distinct species, are clearly of the sort that were to be expected from their hybrid association. It is not fundamental to my position that the various forms of the variants in the F_2 and F_3 generations should match the "mutants" from *Lamarckiana*. Since the F_1 hybrids were not themselves the counterpart of *Lamarckiana*, they should not be expected to give the same progeny as this latter plant. It is sufficient for my purpose to point out the essential parallelism between this hybrid behavior and that of *Lamarckiana* when it gives rise to its "mutations."

De Vries ('12, p. 30) has questioned the stability of my *grandiflora* stock, apparently believing that my hybrids exhibit, at least in part, a mutating habit inherited from the *grandiflora* parent. This view is based on the appearance of two classes of hybrids (twin hybrids) in the F_1 from the cross *grandiflora* *B* \times *biennis* *D*. The evidence, however, indicates that this peculiarity is connected with the *biennis* parent, which may not have been homozygous for the character of stem coloration at the time the cross was made, although in later generations the form has held true.

I am perfectly willing to admit the complexity of my stock material of *grandiflora* and *biennis*, and also the possibility that the forms may not have been strictly homozygous at the time the crosses were made. It was in no wise necessary for the purposes of my experiments that they should be strictly homozygous. My only concern was that the material should be American types of *Ænothera* without the possibility of contamination through crosses with *Lamarckiana*. That my forms of *biennis* and *grandiflora* had these qualifications there can, I think, be no doubt. They have, as a matter of fact, bred true in the small cultures which have been carried through two generations for *biennis A* and *biennis D* and four generations for *grandiflora B*.

An abstract of my argument is as follows: (1) Since hybrids of *biennis* and *grandiflora* show points of strong resemblance to *Lamarckiana* and, (2) since the behavior of these hybrids in the F_2 and F_3 parallel closely the behavior of *Lamarckiana* when it gives rise to "mutants," (3) therefore, there are strong reasons for believing that the "mutations" of *Lamarckiana* are due to instability of its germinal constitution resulting from a hybrid origin. The fact that *Ænothera Lamarckiana* is not known as a component of any native *Ænothera* flora and the fact that its known history has been entirely as a cultivated plant or as a garden escape naturally greatly strengthen the force of the above argument.

It does not seem to me that these arguments are answered by a supposition that the behavior of my hybrids involves a habit of mutation inherited from the parental types. On the contrary, are we not justified in asking of the mutationists evidence from material the status of which, as representative of a wild species, is beyond question? Stomps ('12b) has apparently endeavored to meet the situation by a study of a cross between the *biennis* and *cruciata* of the sand dunes of Holland. From the cross he obtained in the second generation a *biennis nanella* and a *biennis semi-gigas*. Both of

these new forms are regarded by Stomps as "mutants" in the De Vriesian sense in the belief that *biennis* and *cruciata* have an identical germinal constitution, except for factors that determine floral structure and, therefore, with respect to other characters may be crossed as though they were homozygous. Applying these conclusions to the problem of the status of *Æ. Lamarckiana*, Stomps reasons that since *biennis* mutates and since *biennis* is an older species than *Lamarckiana*, it follows that mutations among the *œnotheras* are older than *Lamarckiana* and consequently the mutations of this species can not be the result of hybridization.

In a recent discussion (Davis, '13) of the conclusions of Stomps I have taken exception to the assumption that his *biennis* and *cruciata* have exactly the same germinal constitution except for floral characters. This I can not believe probable, for the reason that, whatever may be the relation between the two species, they have certainly had a long period of independence. *Cruciata* has never appeared in the extensive cultures of the Dutch *biennis* that have been grown by De Vries and Stomps, and there is no experimental evidence that it has been recently derived from the latter form. From my point of view Stomps really made a cross between two species and obtained two marked variants due to some germinal modification as a result of the cross.

It seems to me fair to ask: Why did Stomps find it necessary to cross *biennis* and *cruciata* to obtain these "mutants" *biennis nanella* and *biennis semi-gigas*? If they have the same germinal constitution except for floral characters, Why should not *biennis* alone or *cruciata* alone give the "mutants"? There is no form of *biennis* better known to the workers in the experimental gardens than this Dutch plant. It is believed to have been on the sand dunes of Holland since pre-Linnæan times and Bartlett ('13) has recently presented strong reasons for believing the plant to be the form known to Linnæus as *Enothera biennis* and consequently to be regarded as

the type-form of the species. No species of *Ænothera* is perhaps so free from suspicion as to its gametic purity. If Stomps can obtain mutations from tested material of the Dutch *biennis* grown in pure lines he will have the basis of a strong argument, but this seems to me lacking in the conclusions drawn from his cross of *biennis* with *cruciata*.

I do not believe it at all probable that the Dutch *biennis* will be found to "mutate" under normal conditions to a degree worthy of serious consideration for the mutation theory of De Vries. The plant has already been made the subject of extensive cultures and its characters are known to a number of workers with *œnotheras*. Yet I am far from taking the stand that environmental conditions may never induce a modification of germinal constitution and still leave the organism vigorous. The possibility of direct modification of germ plasm, independent of sexual mixing, presents one of the most inviting fields of genetical research. However, if such research gives affirmative conclusions we should be most cautious in applying them to the conditions that normally surround a species and to the process of organic evolution.

6. THE PROBLEM OF THE ORIGIN OF *Ænothera* *Lamarckiana* DE VRIES

As stated in the introduction to this paper, we are no longer in our problem of the origin of *Ænothera Lamarckiana* De Vries concerned with Lamarek's plant (*Æ. Lamarckiana* Seringe, 1828) of about 1796. This plant (Davis, '12b) was with little doubt a form of *Æ. grandiflora* Solander, 1789, introduced at Kew in 1778. It had no relation to the cultures of Carter and Company, of about 1860, which were the starting point for the distribution among seedsmen of the plants known in cultivation as *Lamarckiana* (an incorrect determination of Lindley) from some of which De Vries's material was derived.

The historical side of the problem then largely centers on the history and composition of these cultures of Carter and Company. We have the statement of this firm that their seeds were received unnamed from Texas. This suggests that *Lamarckiana* De Vries may have in it the blood of some of the large-flowered *œnotheras* with an upright habit that are known to be present in the southwestern United States. There are a large number of such forms which frequently pass under the name of *œnothera Hookeri* and have not as yet been properly segregated in the experimental garden. I am working with several of these types to determine whether any of them may prove to be more favorable than *grandiflora* as forms to cross with *biennis* in my attempts to synthesize *Lamarckiana* as a hybrid. (See note at end of paper.)

It must, however, be borne in mind that we have at present no confirmatory evidence that such plants as Carter and Company describe or the *Lamarckiana* of De Vries's cultures grow in Texas. It is possible that Carter and Company obtained their plants from some part of England, as from the sand hills of Lancashire, where large-flowered *œnotheras* were reported at dates earlier than 1860 and where at the present day *œ. Lamarckiana* is successfully established. We must look to British botanists for investigations which will make clear the history of such *œnothera* floras as that of Lancashire, and it is to be hoped that collections will be thoroughly searched for evidence on their probable development.

With respect to the composition of the cultures of Carter and Company we have some strong evidence from the specimens grown by Asa Gray in 1862 that their plants differed in some important respects from the *Lamarckiana* of De Vries. These specimens have been figured and described (Davis, '12a, pp. 417-422) and it seems probable that the plants were not more than one or two generations removed from the original cultures of Carter and Company. The specimens have characters in part those of De Vries's *Lamarckiana* and in part those

found in *grandiflora*, and undoubtedly present in some of the large-flowered *œnotheras* of the west and southwest. If the plant of Dr. Gray was representative of the cultures of Carter and Company the evidence indicates that their forms became greatly modified during the quarter century before the time when De Vries began his studies, at about 1886, and isolated the type which we know to-day as *Enothera Lamarckiana* De Vries.

On the experimental side of the problem of the origin of De Vries's *Lamarckiana* we have evidence of its hybrid nature from various sources. The recent analytical studies of Heribert-Nilsson ('12), previously mentioned, show that certain characters of *Lamarckiana* behave in a manner suggesting their association in a complex hybrid that is still throwing off segregates. His conclusions that *Lamarckiana* is a polymorphic species is supported by my own experience in isolating certain biotypes from material of De Vries. The "twin hybrids" produced when *Lamarckiana* or certain of its derivatives furnish the pollen of a cross with *biennis* or *muricata* indicate, as suggested by several critics, that different types of gametes are formed by *Lamarckiana*.

My own studies on hybrids between forms of *biennis* and *grandiflora* have reached an interesting point. I have not been able to synthesize by direct crosses, from wild stock so far obtained, any hybrid with all of the characters of *Lamarckiana* in the same plant, although I believe that all of the important taxonomic characters of *Lamarckiana* have been represented in some of my hybrids. It is, however, probable that more favorable parental types will in time come to hand. For example, a form, with the habit and foliage of the Dutch *biennis* and with the stem coloration of *Lamarckiana*, which the Dutch *biennis* apparently has not, would furnish very favorable material. In the meantime I have the possibility of crossing my hybrids back with certain wild species and of crossing the hybrids with one another. In this way it may be possible to bring into one plant all of

the characters of *Lamarckiana*. It is of course in no way essential to the hypothesis of the hybrid origin of *Lamarckiana* that the plant should have arisen as the product of a simple cross. With Lamarck's plant eliminated from the problem of the origin of De Vries's material, the importance of *grandiflora*, on historical grounds, is materially lessened and we may consider other large-flowered types of more recent introduction into Europe as possible parents in a cross.

The resemblance of my various hybrids to *Lamarckiana* and the parallelism of their behavior in the F_2 and F_3 to that of *Lamarckiana* give in themselves sufficient reasons, in my opinion, to justify the belief in its hybrid character and to point to the probability that this plant arose as a cross between distinct forms of *Oenothera*. *Lamarckiana* thus would not be representative of a wild species of essentially stable germinal constitution and its "mutations" are most simply interpreted as the behavior of a hybrid.

UNIVERSITY OF PENNSYLVANIA,
April, 1913

LITERATURE CITED

- Bartlett, H. H., 1913. The Delimitation of *Oenothera biennis* L. *Rhodora*, Vol. XV, p. 48, 1913.
- Davis, B. M., 1910. Notes on the Behavior of Certain Hybrids of *Oenothera* in the First Generation. *AMER. NAT.*, Vol. XLIV, p. 108, 1910.
- Davis, B. M., 1911. Some Hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. *AMER. NAT.*, Vol. XLV, p. 193, 1911.
- Davis, B. M., 1912a. Further Hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. *AMER. NAT.*, Vol. XLVI, p. 377, 1912.
- Davis, B. M., 1912b. Was Lamarck's Evening Primrose (*Oenothera Lamarckiana* Seringe) a form of *Oenothera grandiflora* Solander? *Bull. Tor. Bot. Club*, Vol. XXXIX, p. 519, 1912.
- Davis, B. M., 1913. Mutations in *Oenothera biennis* L.? *AMER. NAT.*, Vol. XLVII, p. 116, 1913.
- De Vries, Hugo, 1901. *Die Mutationstheorie*. Leipzig, 1901-03.
- East, E. M., 1912. The Mendelian Notation as a Description of Physiological Facts. *AMER. NAT.*, Vol. XLVI, p. 633, 1912.

- East, E. M., and Hays, H. K., 1912. Heterozygosis in Evolution and Plant Breeding. Bull. No. 243, Bu. Pl. Ind., U. S. Dept. of Agri., 1912.
- Gates, R. R., 1913. A Contribution to a Knowledge of the Mutating *Enotheras*. *Trans. Linn. Soc. Botany*, Vol. VIII, p. 1, 1913.
- Heribert-Nilsson, N., 1912. Die Variabilität der *Enothera Lamarckiana* und das Problem der Mutation. *Zeitsch. f. ind. Abstam. u. Vererbungslehre*, Vol. VIII, p. 89, 1912.
- Lamarck, ?1798. *Encyclopédie Méthodique Botanique*, Vol. IV, p. 554, ?1798.
- Lutz, Anne M., 1912. Triploid Mutants in *Enothera*. *Biol. Centralbl.*, Vol. XXXII, p. 385, 1912.
- Seringe, N. C., 1828. De Candolle, *Prodromus*, Vol. III, p. 47, 1828.
- Solander, D., 1789. Aiton, *Hortus Kewensis*, Vol. II, p. 2, 1789.
- Stomps, T. J., 1912a. Die Entstehung von *Enothera gigas* De Vries. *Ber. deut. bot. Gesell.*, Vol. XXX, p. 406, 1912.
- Stomps, T. J., 1912b. Mutation bei *Enothera biennis* L. *Biol. Centralbl.*, Vol. XXXII, p. 521, 1912.

NOTE ADDED AUGUST 10, 1913

It is a satisfaction to announce that this summer (1913) I have obtained an F₁ hybrid generation with, I believe, all of the essential taxonomic characters of the small-flowered forms of *Æ. Lamarckiana* De Vries. The cross was a large-flowered, undescribed species of *Enothera* from California pollinated by the Dutch *biennis* (*Æ. biennis* Linnaeus). The hybrids differ from the small-flowered *Lamarckiana* only in relatively small plus or minus expressions of certain of its distinctive characters. It is not unreasonable to expect that generations from these hybrids in the F₂ will give material for future selection towards the large-flowered *Lamarckiana* of De Vries.

SHORTER ARTICLES AND DISCUSSION

NOTES ON A DIFFERENTIAL MORTALITY OBSERVED BETWEEN TENEBRIO OBSCURIS AND *T. MOLITOR*

I RECENTLY had occasion to subject some meal worms for short periods of time to a temperature considerably higher than that of the laboratory. Although the experiment was begun with a different purpose in view, there has been one feature noted which seems worth recording at this time.

The meal worms used consisted of the larvæ of *Tenebrio molitor* Linn. and *T. obscuris* Fabr. These larvæ are very readily distinguished from each other by the fact that the pigment in the integuments of *T. molitor* is yellow to orange-brown, while that of *T. obscuris* is almost black. In other features the larvæ resemble each other to a remarkable extent.

EXPERIMENTS WITH ELEVATED TEMPERATURE

The worms were placed in large Stender dishes containing a little meal and the dishes were then placed in a large, constant temperature incubator, being insulated from the bottom of the incubator by a cork ring and care being taken that the glass sides of the dishes did not come in contact with the copper sides of the incubator. The temperature was frequently noted through the glass door of the incubator and was also recorded on a maximum and minimum thermometer placed inside of the incubator and likewise insulated from contact with the copper sides or floor.¹

Three major experiments were made after a probable differential mortality had been observed. These experiments are summarized below.

¹ A much higher temperature is obtained if contact with the walls or floor is allowed. When an experimental dish was not insulated from the floor all of the larvæ which it contained were dead when larvæ in an insulated dish were still active.

Experiment 1.—104 larvæ (53 *T. molitor* and 51 *T. obscuris*). Kept at a temperature of 43° C. for 3 hours. These were the survivors of a previous heating at 41°–42° for 4 hours in which no record was kept of the number dying. Count made 24 hours after last heating.

	<i>T. molitor</i>	<i>T. obscuris</i>
Normal or nearly normal	2	44
Sluggish	13	6
Barely alive	13	1
Dead	25	none
Per cent. dead	47.17	—

Experiment 2.—50 larvæ (25 *T. molitor* and 25 *T. obscuris*). Heated 3 hours at 43° C. Not previously heated. Count made 24 hours later

	<i>T. molitor</i>	<i>T. obscuris</i>
Normal or nearly normal	none	none
Sluggish	none	none
Barely alive	none	12
Dead	25	13
Per cent. dead	100	52

Experiment 3.—441 larvæ (204 *T. obscuris* and 237 *T. molitor*). Heated at 41.5°–42° for 3.5 hours.

	Count in 24 Hrs.		Count in 48 Hrs.		Count in 96 Hrs.	
	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>
Normal or nearly normal.....	none	176	none	164	none	164
Sluggish.....	none	7	none	7	21	6
Barely alive.....	195	15	160	21	128	10
Dead.....	42	6	77	12	88	24
Per cent. dead.....	17.72	2.94	32.50	5.88	37.14	11.77
Per cent. barely alive.....	82.28	7.35	67.50	10.29	54.01	4.90

Inasmuch as these experiments show a remarkable differential mortality, two other series of experiments were undertaken, in one of which the external influence used was exposure to cold for a long period of time and in the other set, exposure to an atmosphere of pure carbon dioxide.

EXPERIMENTS WITH COLD

Two experiments were made on the influence of cold as affecting the death rate. Two large Stender dishes, each of which contained 50 *T. molitor* and 50 *T. obscuris*, were placed inside of a battery jar, together with a maximum and minimum thermometer, the top of the jar was closed with a sheet of rubberized cloth

to keep out moisture, and the whole was then fastened outside a laboratory window, in such a manner that the bottom rested upon a concrete slab, but the remainder of the jar was not in contact with the laboratory walls. The experiment ran from December 28, 1912, to March 9, 1913.

Unfortunately for this experiment, the winter at Cold Spring Harbor was unusually mild and the minimum temperature recorded in the jar was only -10° C. with a maximum of $+11^{\circ}$.

The jar was then taken into the laboratory and after standing at room temperature for 24 hours a count was made. A second count was made six days later with the results given below.

	First Count				Second Count	
	Experiment 1		Experiment 2		Exps. 1 and 2 Combined	
	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>
Alive.....	40	47	33	44	50	91
Dead.....	10	3	17	6	50	9
Per cent. dead...	20	6	31	12	50	9

EXPERIMENTS WITH CARBON DIOXIDE

In the third set of experiments, the influence of carbon dioxide on the differential mortality, approximately 75 larvæ of each species were placed in each of three gas wash bottles (E. & A. No. 3,658). The wash bottles were connected together with rubber tubing and carbon dioxide from a Kipp generator (limestone and hydrochloric acid) was slowly passed through the apparatus during the entire course of the experiment. The carbon dioxide was first washed through a saturated solution of sodium bicarbonate and then through distilled water before passing to the bottles containing the larvæ.²

The stream of carbon dioxide was started at 12 M., December 19. At 12:05 P.M. the jar nearest the generator (Jar No. 1) showed no movement of *T. molitor* but the *T. obscuris* were still very active; at 12:07 P.M. only a few *T. obscuris* were moving in jar No. 1 and nearly all of the *T. molitor* in jar No. 2 were dormant; at 12:09 all of the *T. molitor* in each of the three jars were in a state of "suspended animation" but a few *T. obscuris* were

² I have already shown (*J. Biol. Chem.*, 10, p. 90, and *AMER. NAT.*, 45, pp. 749-750) that it is possible to keep insects in pure carbon dioxide for hours without causing death, although to all appearances they are dead within a very few minutes after being subjected to the action of the gas.

still moving in jar No. 1; 12:11 P.M. "some movement in each jar but only of *T. obscuris*"; 12:20 P.M. "There are still a few *T. obscuris* moving in each jar, these have bubbles at their mouth"; 12:25 "Still a slight movement of a few but the 'suspended animation' is practically complete"; 12:30 P.M. "No movement in any."

An analysis of the gas passing through the apparatus was made at 3 P.M., December 19, and it was found to consist of 98.92 per cent. by volume of carbon dioxide (absorbed by KOH) and 0.27 per cent. of oxygen (absorbed from the residual gas by alkaline pyrogallol). Another analysis was made at 11 A.M., December 20, showing 99.15 per cent. of carbon dioxide and 0.04 per cent. of oxygen.

At the end of each experiment the last wash bottle of the chain was detached from its mate, a rapid current of air was drawn through it for several minutes, and then the larvæ were shaken out into a large open dish and allowed to remain fully exposed to the air. Counts were made at intervals. The results of the experiment are shown in the table below.

THE EFFECT OF CARBON DIOXIDE ON DIFFERENTIAL MORTALITY

	Jar No. 3		Jar No. 2		Jar No. 1	
Hrs. in CO ₂	23.5		46.5		51.5	
Date Removed	Dec. 20, 11:30 A.M.		Dec. 21, 10:30 A.M.		Dec. 21, 3:30 P.M.	
	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>	<i>T. mol.</i>	<i>T. obs.</i>
Count made Dec. 21, 9 A.M.:						
Alive	66	51				
Dead	10	24				
Count made Dec. 21, 10 A.M.:						
Alive.....			64 ^a	30 ^a	48 ^a	8 ^a
Possibly alive			5	15	21	35
Certainly dead (discolored).....			5	31	6	35
Count made Dec. 28:						
Alive.....	60	38	66	26	59	10
Dead (discolored).....	16	37	9	50	16	68
Per cent. dead.....	21.05	49.33	11.99	65.80	21.33	87.18

SUMMARY

The exposure of larvæ of *Tenebrio molitor* and *Tenebrio obscuris* to elevated temperature for a few hours causes a much greater mortality among the larvæ of *T. molitor* than among those

^a None able to crawl as yet. The feet only are moving.

of *T. obscuris*; 37.14 per cent. of *T. molitor* are dead after 3.5 hours at 42° as contrasted with 11.77 per cent. deaths of *T. obscuris*.

Exposure to cold for a long period of time causes a differential mortality in the opposite direction, nearly all of *T. molitor* (91 per cent.) remaining alive while 50 per cent. of *T. obscuris* died.

Subjecting a mixture of the larvæ to an atmosphere of pure carbon dioxide for 24 to 51 hours causes a differential mortality in favor of *T. molitor*, only 21.33 per cent. dying after 51.5 hours in the carbon dioxide as contrasted with 87.18 per cent. of *T. obscuris*.

It has been my experience, and I understand that owners of bird stores have noted the same fact, that there is a relatively high death rate among the larvæ of *T. obscuris* under natural conditions, while almost none of the larvæ of *T. molitor* die before pupating.

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